Modern Mass Spectrometry-Based Platforms Solving Complex Biological Question

Epigenetics and Cancer - Histone Modifications

Samples – Preparation – Separation – Mass Spec Analysis – Informatics – Reporting

Archive Presentation – November 2011

Basic Histone Epiproteomics Assays

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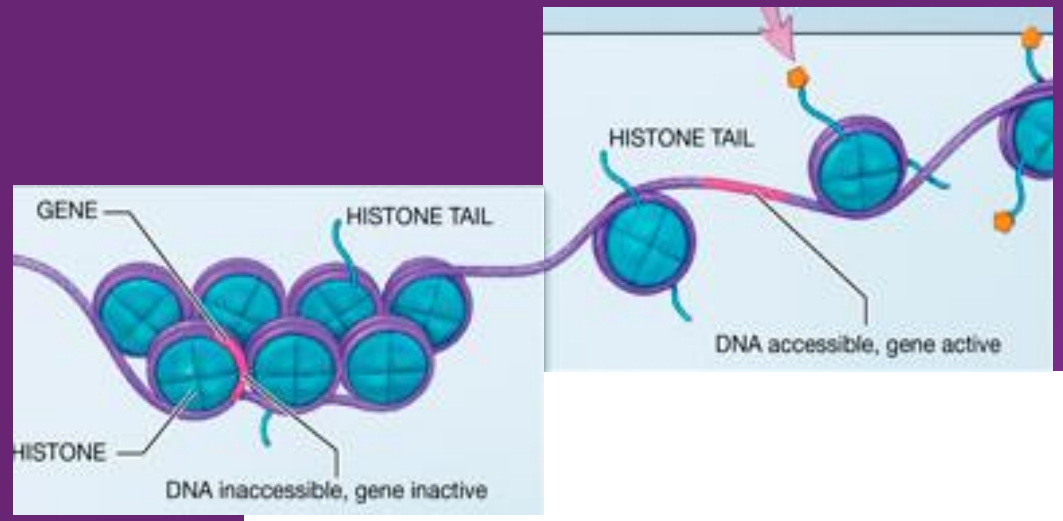
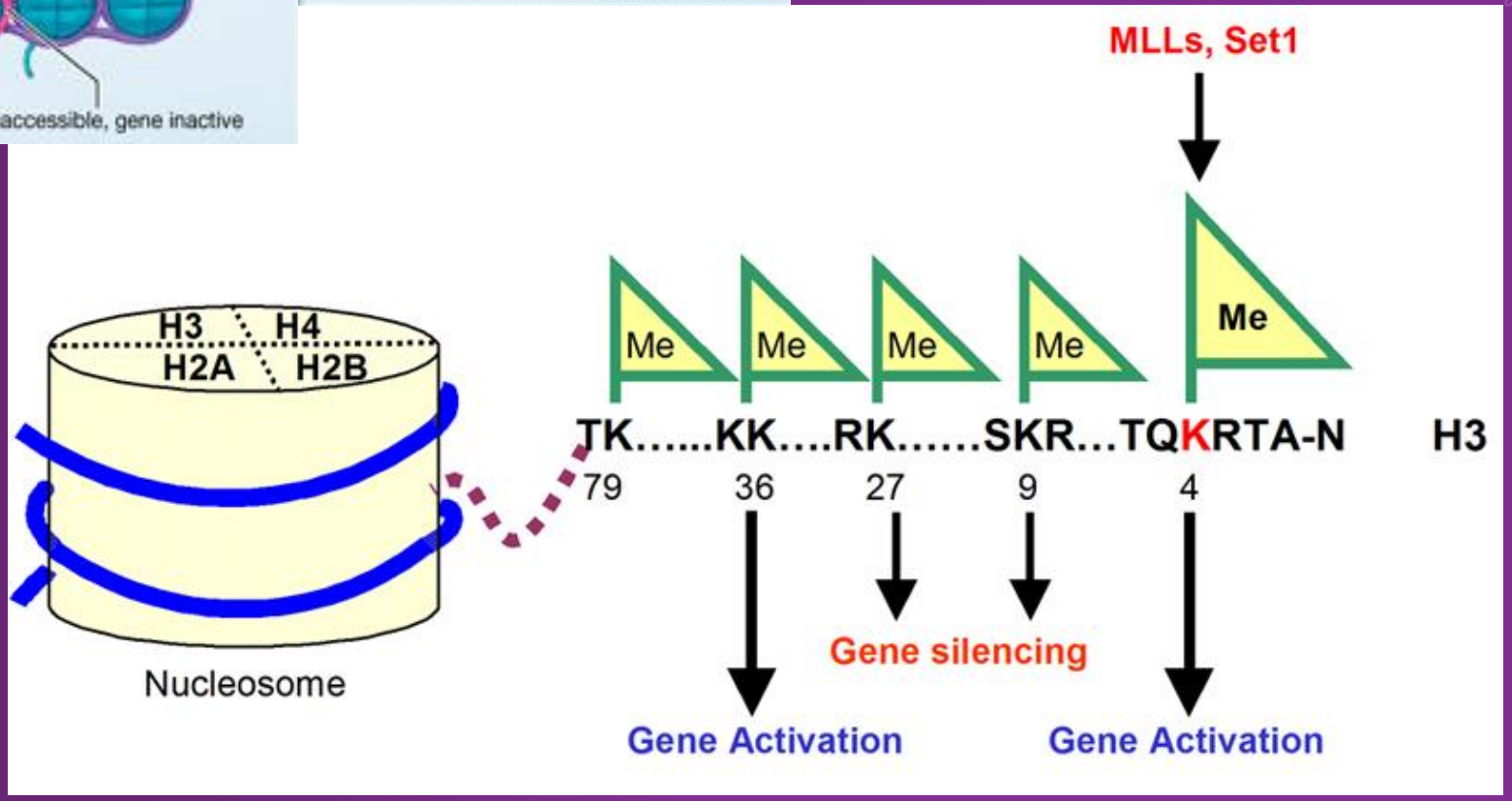
Epigenetics and Cancer - Histone Modifications



Histone Acetyl Transferase (HAT)
Histone Deacetylase (HDAC)
Histone Methyltransferase



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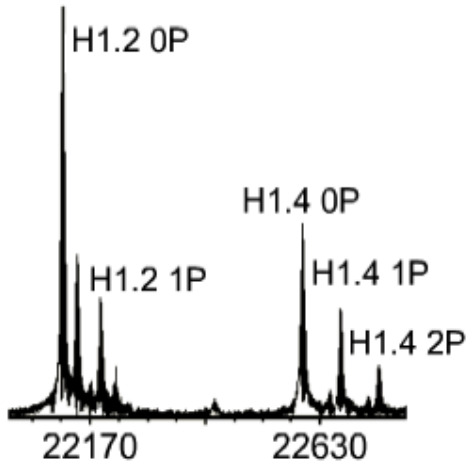
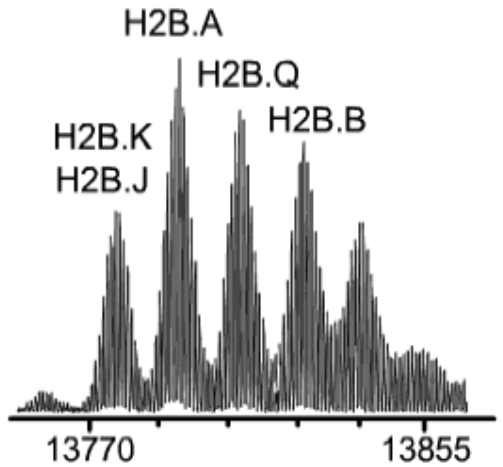
Epigenetics and Cancer - Histone Modifications

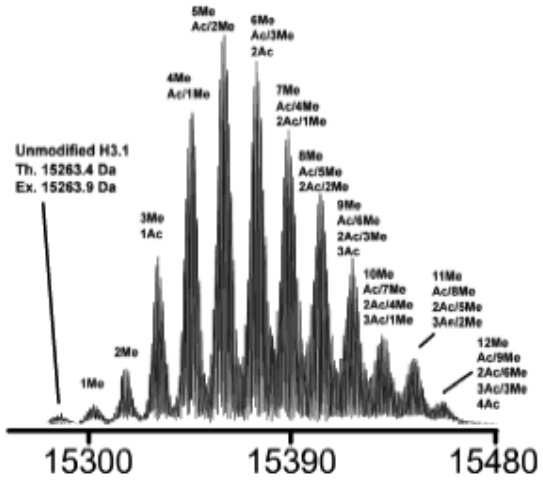
(2011)

Type of Modification	H3K4	H3K9	H3K14	H3K27	H3K79	H3K20	H2BK5
Mono-methylation	activation	activation		activation	activation	activation	activation
Di-methylation		repression		repression	activation		
Tri-methylation	activation	repression		repression	repression		repression
Acetylation		activation	activation				

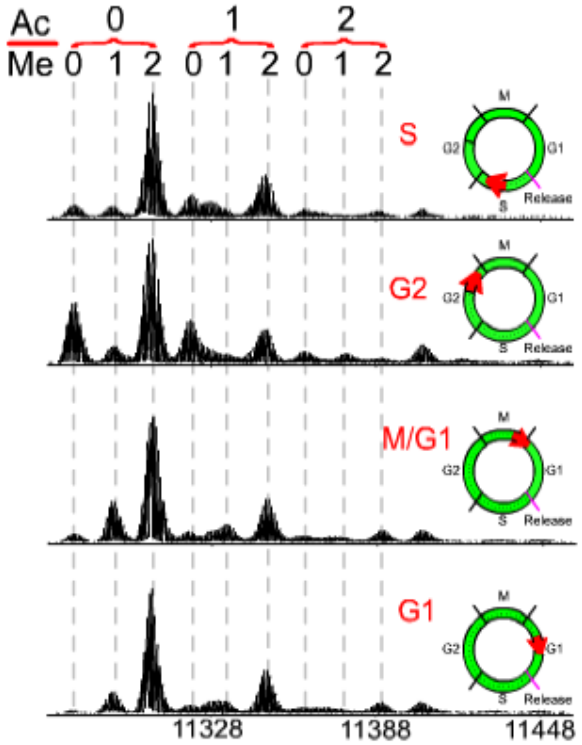
Epigenetics and Cancer - Histone Modifications



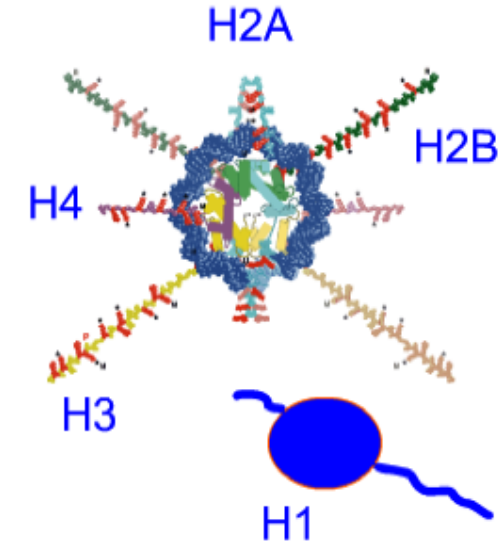


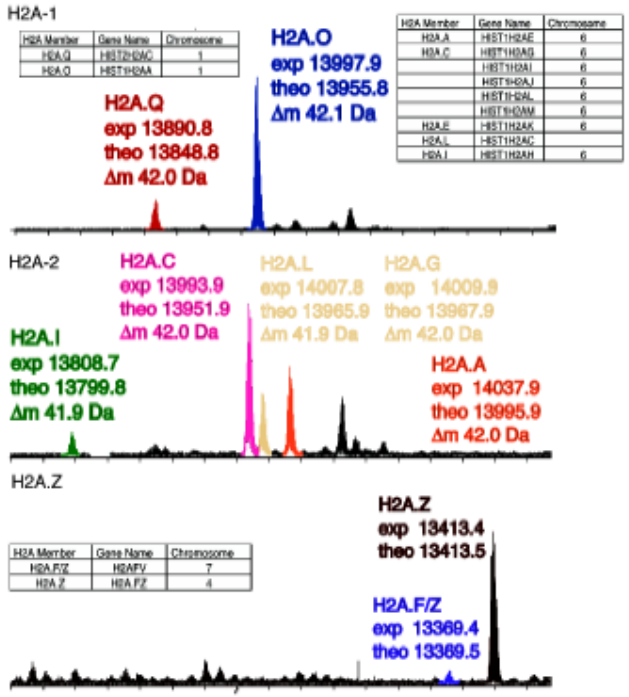


H4 PTM Dynamics During Cell Cycle

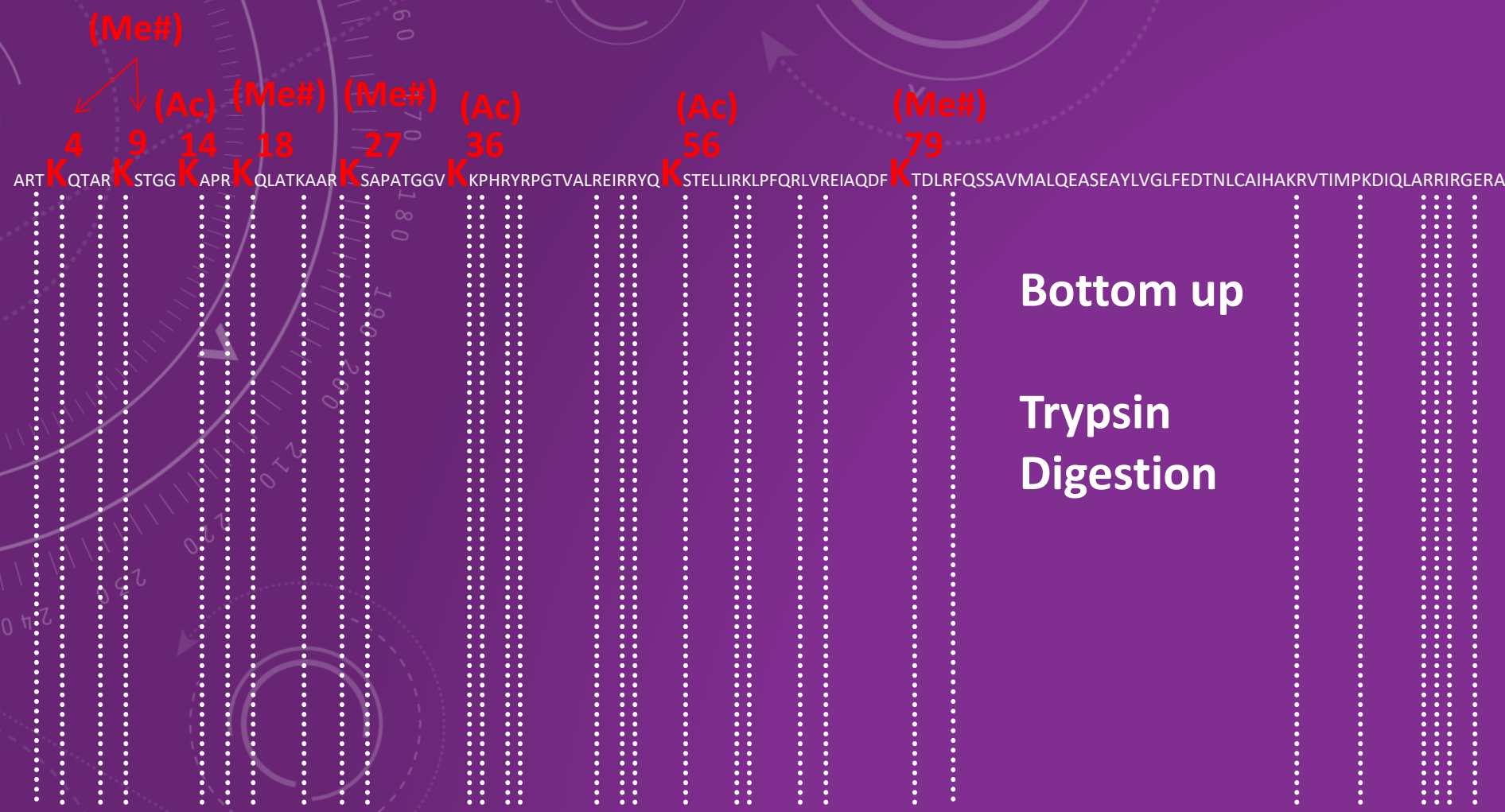


Characterization of H2A Gene Family Members





Histone LCMS - QqQ Assay - Basic Assay



Other Common Strategies Developed – Glu C /Asp N/Arg C and chemical derivatization.

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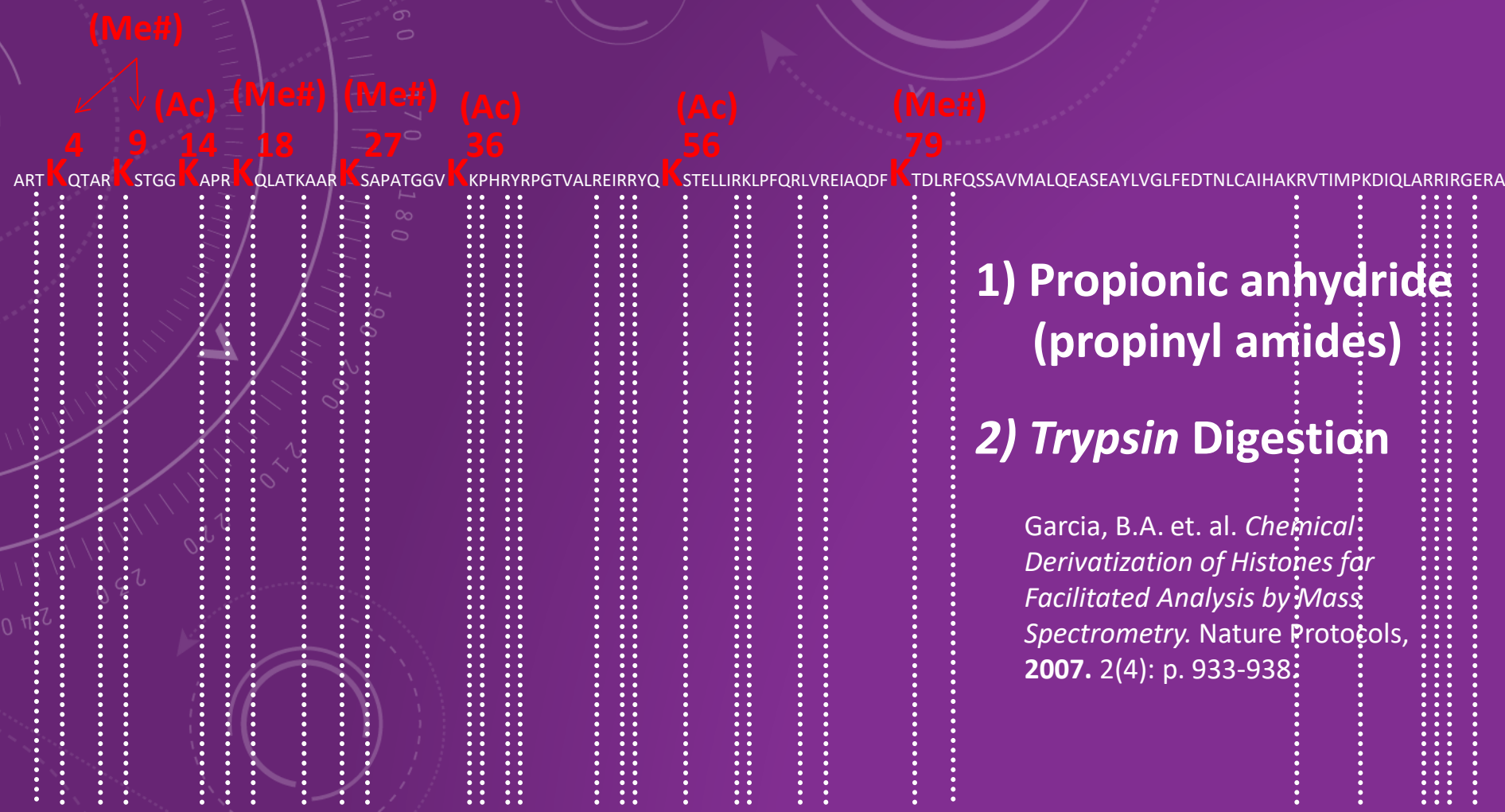
Histone LCMS - QqQ Assay - Basic Assay



1) Propionic anhydride (propinyl amides)

***2) Trypsin* Digestion**

Garcia, B.A. et. al. *Chemical Derivatization of Histones for Facilitated Analysis by Mass Spectrometry.* Nature Protocols, **2007.** 2(4): p. 933-938.

K4 Peptide / K9 K14 Peptide / K18 Peptide / K27 K36 Peptide / K 56 Peptide / K79 Peptide

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Histone LCMS - QqQ Assay - Basic Assay
Assay Development – "K9-K14" Peptide

*255.170 me 1 K S T G G x K A P R 829.453	(2+) 542.3115	(2+) 535.3063	255.170 me 1 ac K S T G G K A P R 815.442
213.158 me2 K S T G G x K A P R 829.453	521.3055	514.3004	213.158 me2 ac K S T G G K A P R 815.442
* 227.175 me 3 K S T G G x K A P R 829.453	528.3140	521.3089	227.175 me 3 ac K S T G G K A P R 815.442
227.175 *ac K S T G G x K A P R 829.453	528.2985	521.2934	227.175 ac ac K S T G G K A P R 815.442
241.145 *x K S T G G x K A P R 829.453	535.3036	528.2985	241.145 x ac K S T G G K A P R 829.442

Histone LCMS - QqQ Assay - Basic Assay
Assay Development – “K9-K14” Peptide

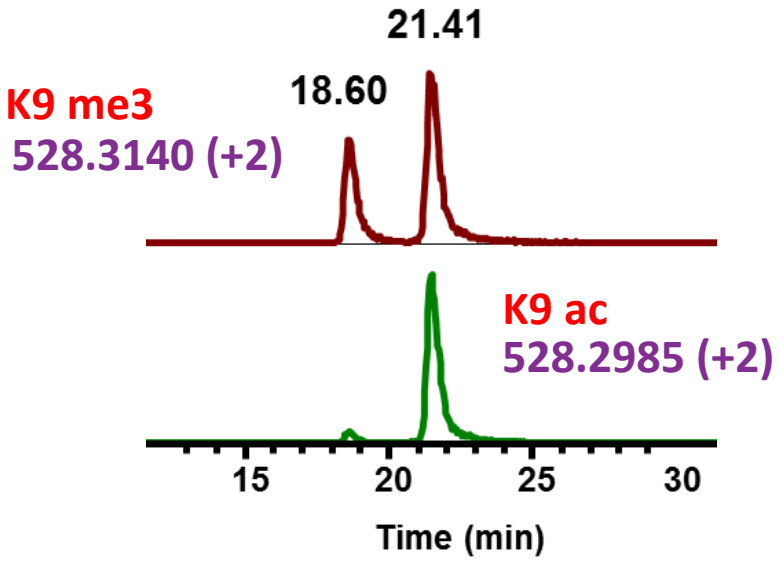
Add signal response for all K9/K14 peptides
(Response in the peak area)

Divide the individual peak area by the total area
(Result is the Percent Relative Occupancy)

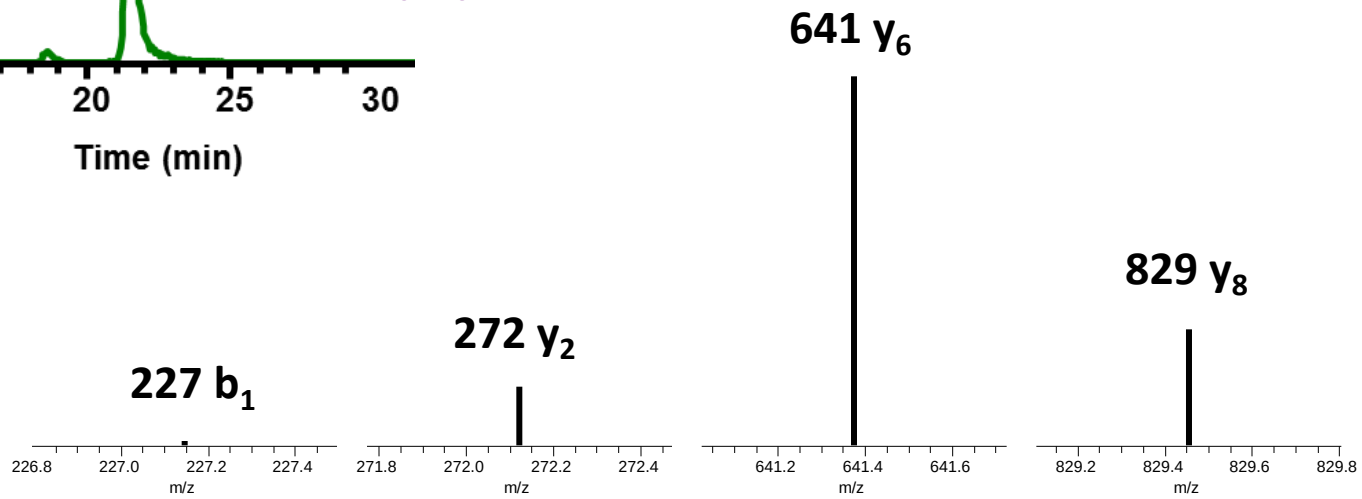
Final Result is a ‘Digital Western Blot ‘

Histone LCMS - QqQ Assay - Basic Assay

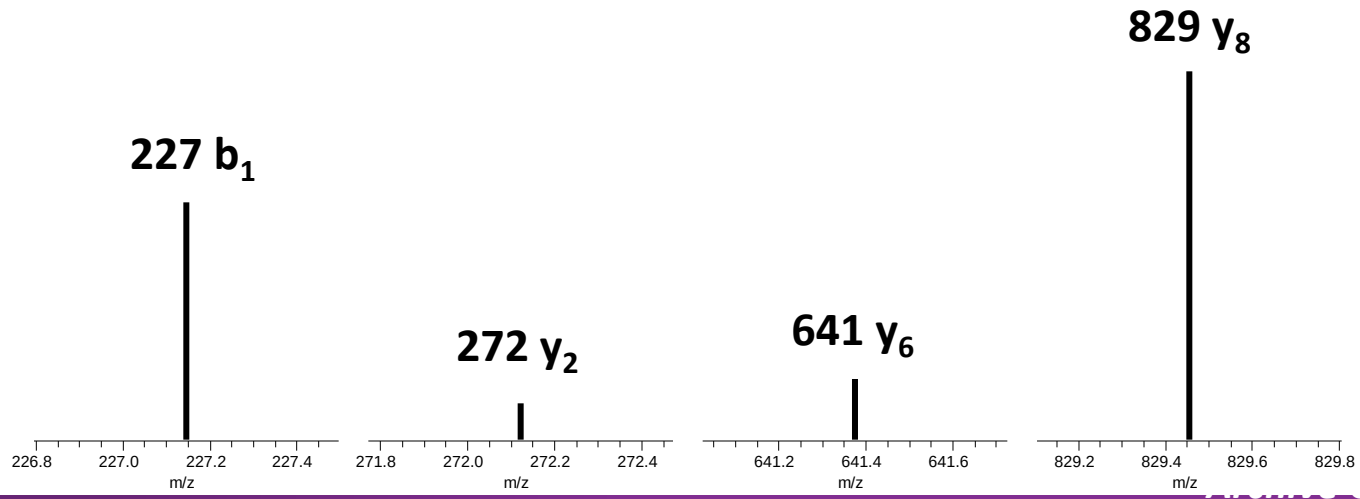
Technical Note and Understanding



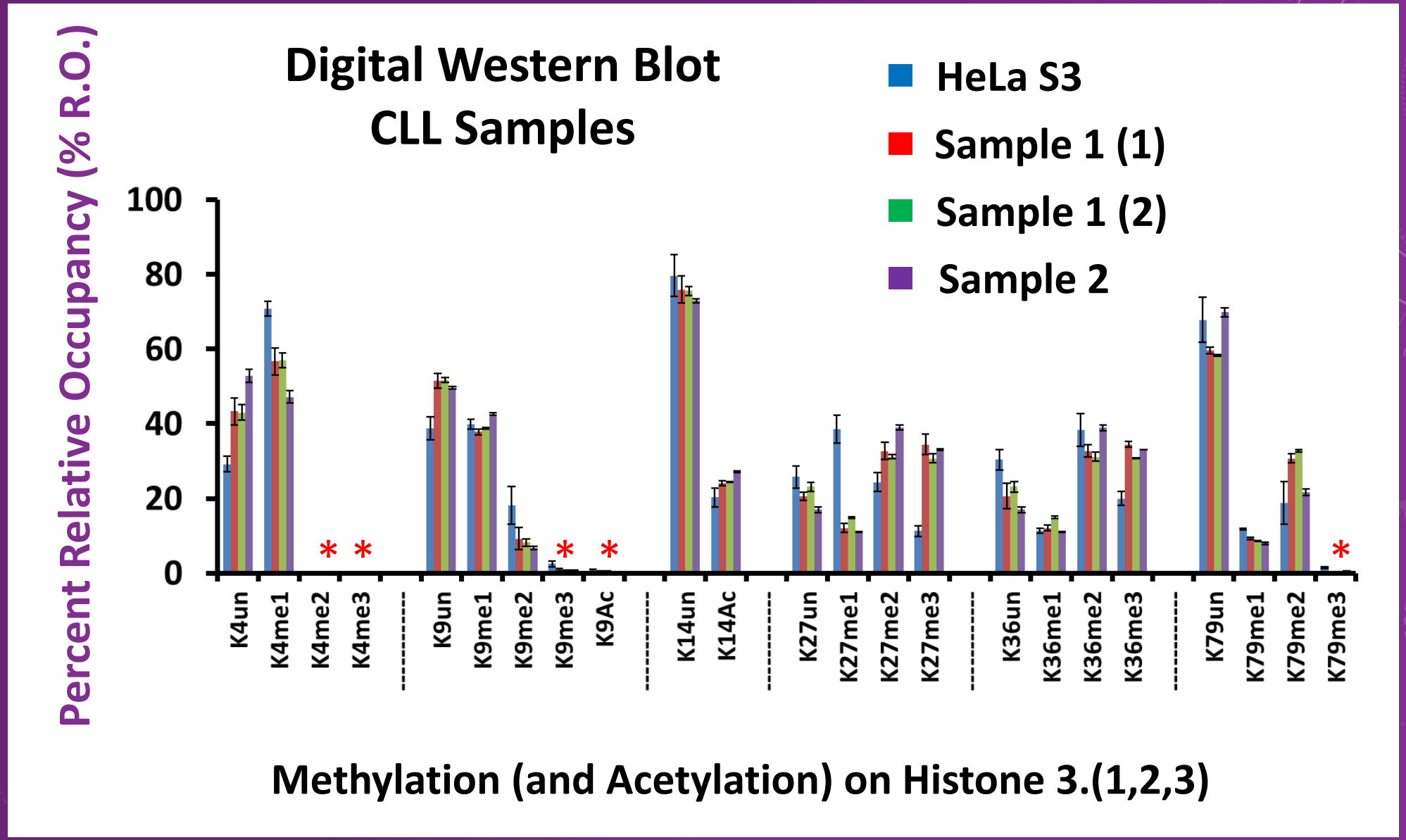
K9 me3



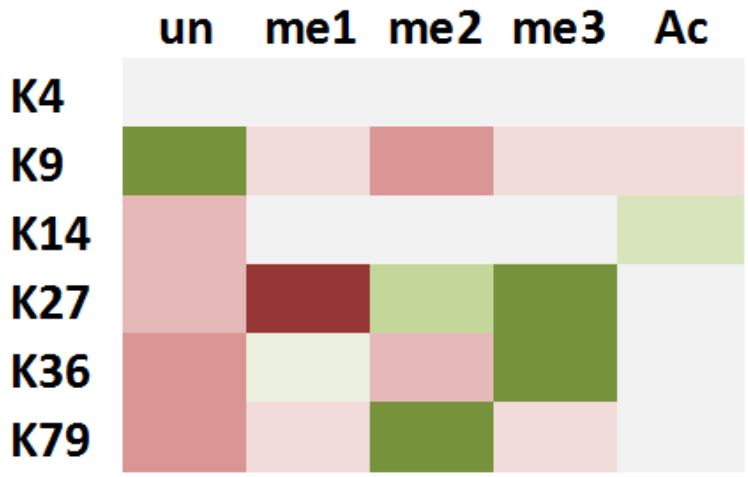
K9 ac



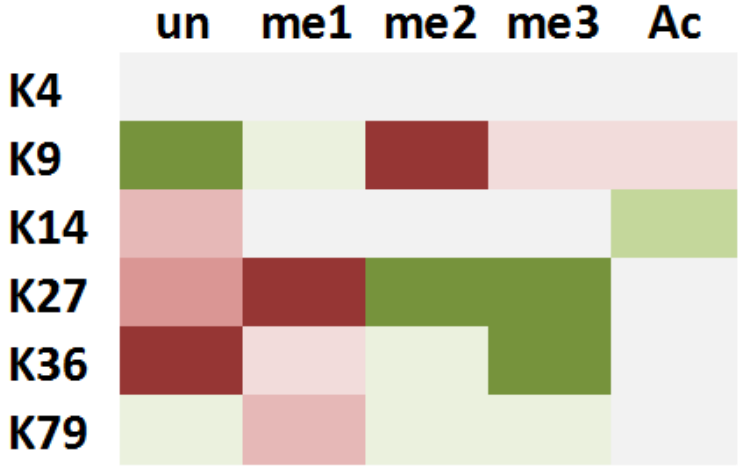
Histone LCMS - QqQ Assay - Basic Assay - Results



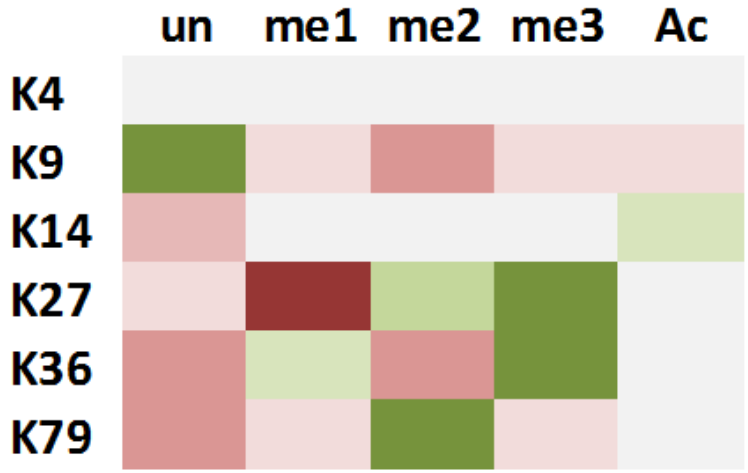
Histone LCMS - QqQ Assay - Basic Assay - Results



Patient 1 (Sample 1)



Patient 2



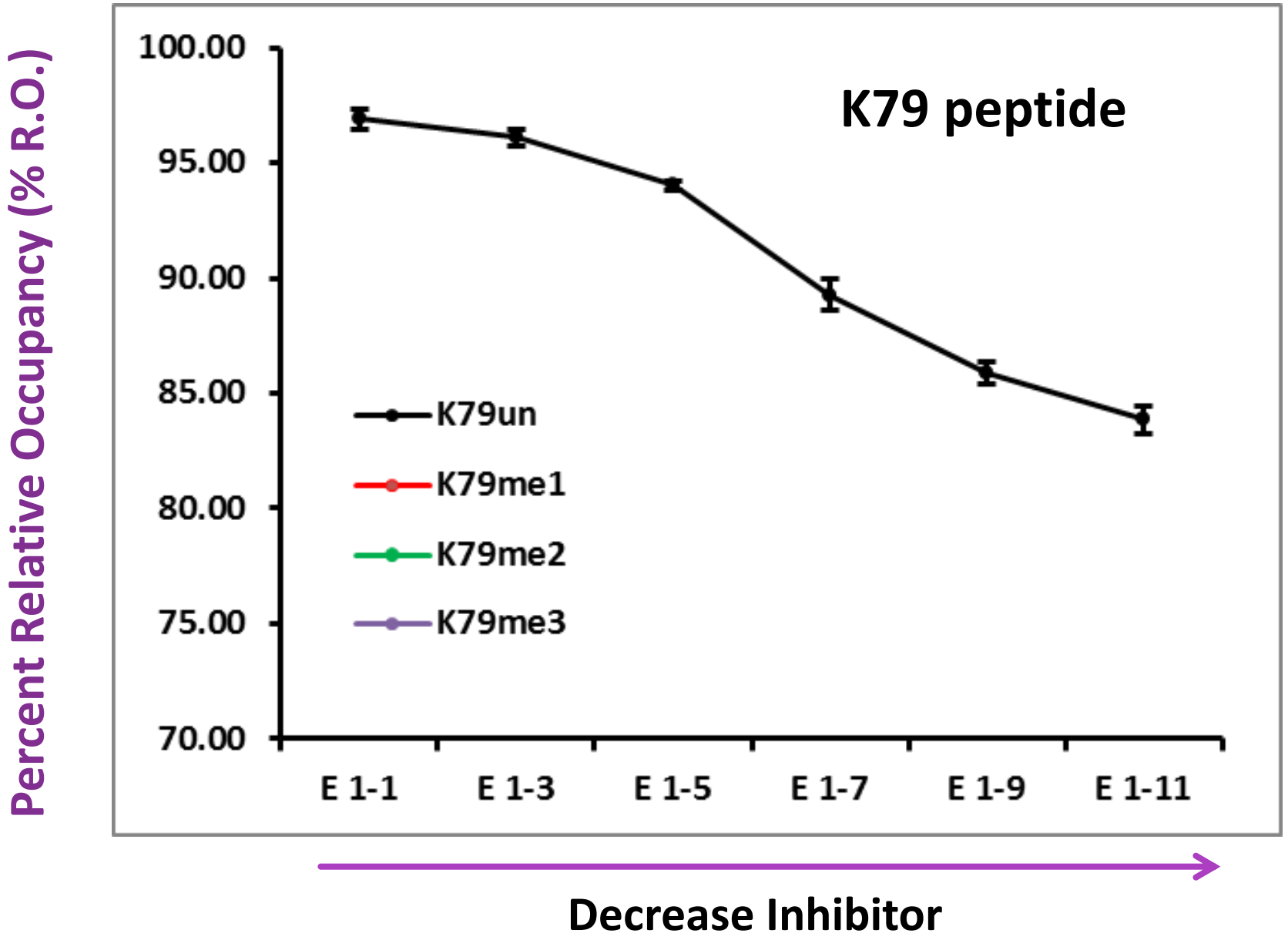
Patient 1 (Sample 2)

Reference for "Heat Map"
Patient Sample - HeLa S3

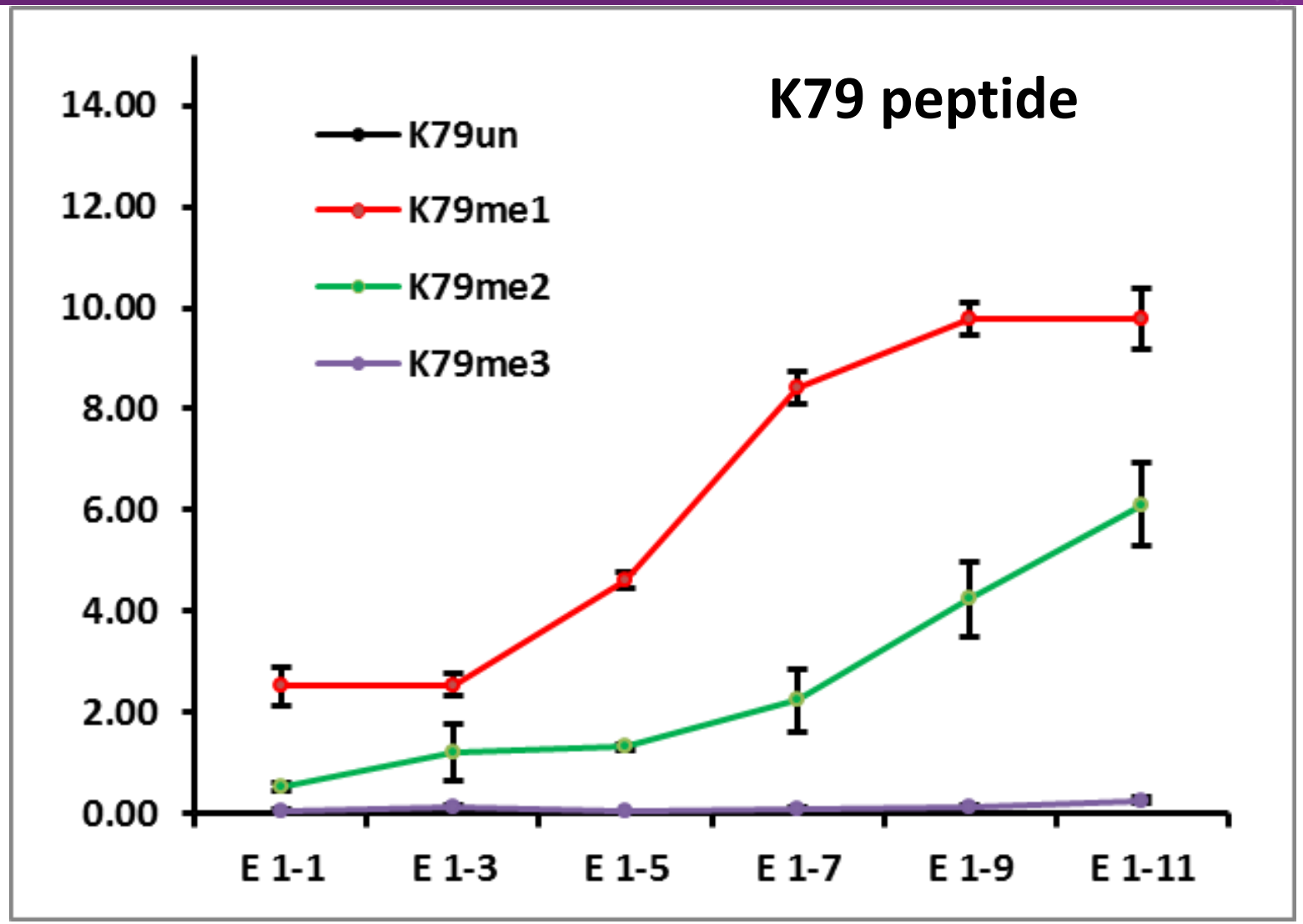
Delta %R.O.	(-) regulation	(+) regulation
0 - 2 %		
2 - 5 %		
5 - 10 %		
10+ %		

Data from Oncology Samples

Histone LCMS - QqQ Assay - Basic Assay - Results

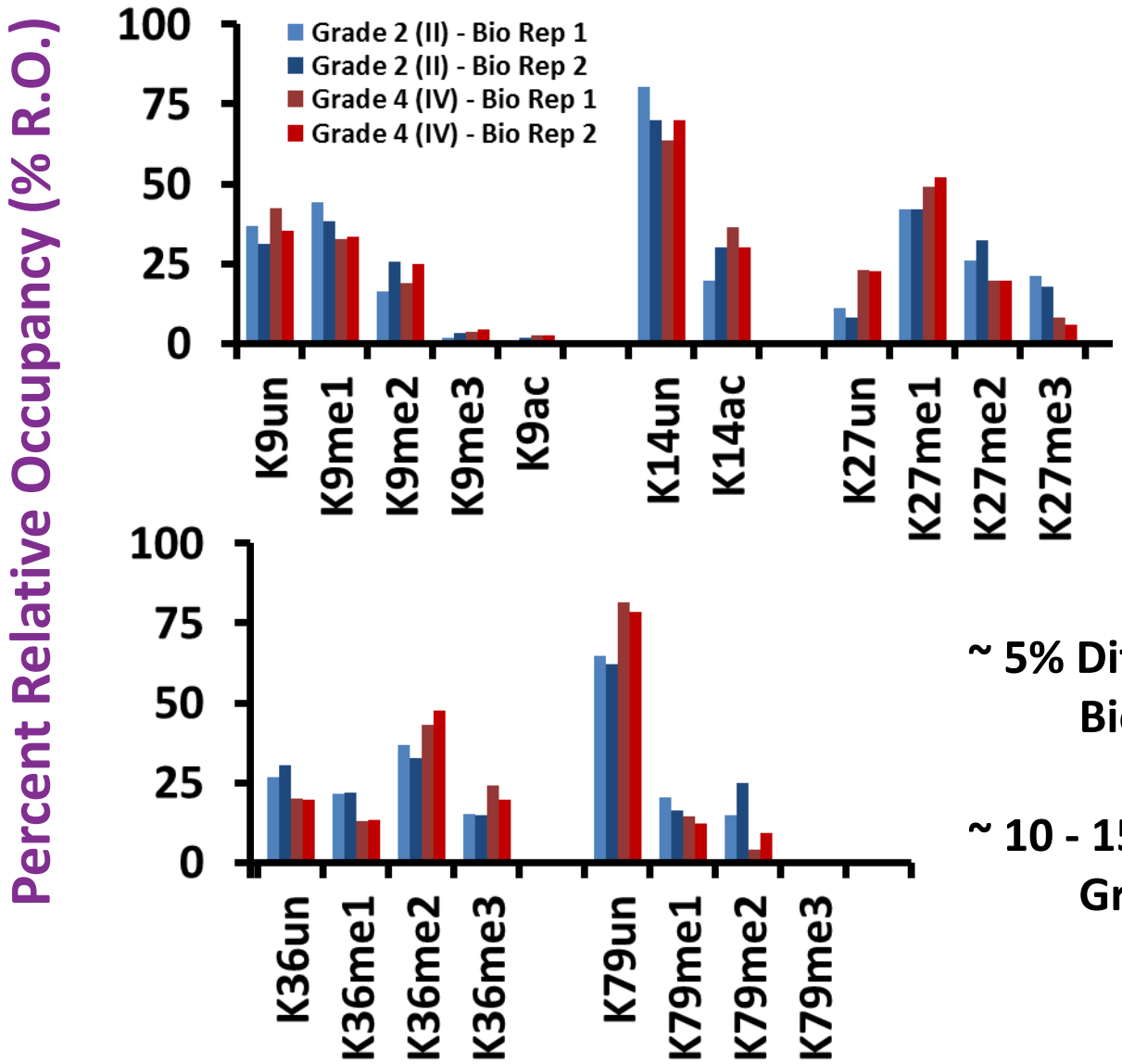


Histone LCMS - QqQ Assay - Basic Assay - Results



Histone LCMS - QqQ Assay - Basic Assay - Results

Oncology Samples



2-5 % Coefficient of Variance Between Technical Triplicates

~ 5% Difference Between Biological Reps

~ 10 - 15% Difference Between Grade II and IV Cells

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